

Chemical Name: Afidopyropen
USEPA PC Code: 026200
USEPA MRID: 49689112
USEPA DP Barcode: 435146
PMRA Data Code (DACO): 9.9
PMRA Study No. (UKID): 2628025
Data Requirement: Non-guideline; OECD Guideline No. 232

Test Material: BAS 440 I (TGAI, INSCALIS™)

Purity: 94.54%

Active Ingredient: Afidopyropen

IUPAC Name: [(3*S*,4*R*,4*aR*,6*S*,6*aS*,12*R*,12*aS*,12*bS*)-3-(cyclopropylcarbonyloxy)-1,2,3,4,4*a*,5,6,6*a*,12*a*,12*b*-decahydro-6,12-dihydroxy-4,6*a*,12*b*-trimethyl-11-oxo-9-(3-pyridyl)-11*H*,12*H*-benzo[*f*]pyrano[4,3-*b*]chromen-4-yl]methylcyclopropane carboxylate
CAS Name: [(3*S*,4*R*,4*aR*,6*S*,6*aS*,12*R*,12*aS*,12*bS*)-3-(cyclopropylcarbonyloxy)]-1,3,4,4*a*,5,6,6*a*,12,12*a*,12*b*-decahydro-6,12-dihydroxy-4,6*a*,12*b*-trimethyl-11-oxo-9-(3-pyridyl)-2*H*,11*H*-naphtho[2,1-*b*]pyrano[3,4-*e*]pyran-4-yl]methylcyclopropanecarboxylate
CAS No.: 915972-17-7
Synonyms: INSCALIS™

Primary Reviewer: Moncie V. Wright
Environmental Scientist, CDM Smith/CSS-Dynamac JV

Signature: 

Date: 17 January 2017

Secondary Reviewer: John Marton, Ph.D.
Environmental Scientist, CDM Smith/CSS-Dyanmac JV

Signature: 

Date: 18 January 2017

USEPA Reviewer: Cameron Douglass, Ph.D.
Biologist, USEPA/OCSP/OPP/EFED/ERBIV

Signature:  2018.02.16 14:49:24 -05'00'
Date: 16 February 2018

PMRA Reviewer: Vedad Izadi
Evaluation Officer, PMRA/EAD/ERSII

Date: 18 August 2017

Date Evaluation Completed: 18 August 2017

Note that the USEPA reviewer verified that statistical analyses (if appropriate) were correctly performed, but did not comprehensively revise the summary document prepared by CDM/CSS-Dynamac JV personnel ("the contractor"). The USEPA reviewer confirmed reported study author endpoints, but took at 'face value' the contractor's summary of the original study report.

CITATION:

S Friedrich. 2015. Effect of BAS 440 I (Reg. No. 5599022, ME5343 Technical) on the Reproduction of the Collembolan *Folsomia candida*. Study conducted by BioChem agrar GmbH, Gerichshain, Germany.

Laboratory study number: 15 10 48 232 S. Study sponsored by BASF SE, Ludwigshafen, Germany. Study initiated August 14, 2015 and completed October 29, 2015.

Executive Summary:

A 28-day reproduction study was conducted with 9-12 day old juveniles of *Folsomia candida*, which were exposed to BAS 440 I (TGA1 afidopyropen) at nominal concentrations of 0 (solvent control), 8.2, 14.7, 26.5, 47.6, 85.7, 154.3, 277.8, and 500 mg a.i./kg soil dry weight.

All collembolans in the control and treatment groups exhibited similar behavior during the test. Survival in the solvent control averaged 96%. Survival ranged from 93 to 100% across all treatment groups except for the 277.8 and 500 mg a.i./kg dw soil groups where survival averaged 75 and 28%, respectively. The number of juveniles produced averaged 700 in the solvent control. Juvenile production ranged from 681 to 705 across all treatment groups except for the 277.8 and 500 mg a.i./kg dw soil groups where juvenile production averaged 454 and 310, respectively.

There were significant ($p < 0.05$) treatment related reductions in both collembolan survival and reproduction.

Results Synopsis:

Survival:

LC ₅₀ : 386 mg a.i./kg dw soil	95% C.I.: 343-434 mg a.i./kg dw soil
Slope: N/A	95% C.I.: N/A
NOAEC: 154.3 mg a.i./kg dw soil	LOAEC: 277.8 mg a.i./kg dw soil

No. of Offspring:

EC ₅₀ : 426 mg a.i./kg dw soil	95% C.I.: 346-N/A mg a.i./kg dw soil
Slope: 0.75	95% C.I.: 0.38-1.13
NOAEC: 154.3 mg a.i./kg dw soil	LOAEC: 277.8 mg a.i./kg dw soil

Endpoint(s) affected: Survival and no. of offspring

EPA Classification: Supplemental (may be used quantitatively for risk estimation)

PMRA Classification: Fully reliable

I. DATA SOURCE

USEPA MRID No.:	49689112
PMRA UKID:	2628025
Study Title:	Effect of BAS 440 I (Reg. No. 5599022, ME5343 Technical) on the Reproduction of the Collembolan <i>Folsomia candida</i> .
Study Author(s):	S Friedrich
Testing Laboratory:	BioChem agrar GmbH, Gerichshain, Germany
Laboratory Report No.:	15 10 48 232 S
Sponsor Study No.:	394892
Study Completion Date:	October 29, 2015
Data Access:	Data submitter is data owner
Data Protection Claimed:	Yes; no claim of confidentiality was made.

II. MATERIALS AND METHODS

Test Guideline: Currently, no U.S. EPA guidance exists; OECD Guideline No. 232

Deviations from Guideline:

This was an EPA non-guideline study, but it adhered to OECD No. 232. The reviewer used the updated guideline adopted July 29, 2016 to assess the study methods. No deviations from OECD No. 232 were noted by the reviewer. However, there were two deficiencies according to standard EPA scientific practices for ecotoxicity testing:

- 1.) A negative control was not included in the study; and
- 2.) Analytical verification of the treated artificial soil was not performed.

GLP Compliance: Yes; study conducted in compliance with the OECD and German Principles of Good Laboratory Practice, and meets the U.S. EPA GLP Standards (40 CFR Part 160 and 792), with the exception that recognized differences exist between the GLP Principles/Standards of OECD and those of the EPA.

A. MATERIALS

Test Material: BAS 440 I (TGAI, INSCALIS™), purity of 94.54%

Test Material Identity: Batch no. 080722

Details on Preparation and Application of Test Materials:

Test included a solvent control (acetone) only. The test material was dissolved in acetone and mixed into finely ground quartz sand. The acetone was evaporated in a fume hood for ~60 minutes. The stock mixture was diluted with quartz sand such that 10 g of the mixture would contain the amount of test material required for one treatment group to adjust the selected concentration. The treated quartz sand (10 g per treatment group) was added to the prepared amount of artificial soil (302.5 g wet weight). The resulting mixture was mixed thoroughly by intensive stirring in a laboratory mixer. A solvent control was prepared in the same manner, only without the addition of the test material at the first step of the process.

Analytical Monitoring: Analytical verification of the test material in the dosing solutions was not performed.

Details on Analytical Method: N/A

Reference Material: Boric acid (100%)

Reference Material Identity: Not reported

Vehicle: Acetone

Test Organism (Species): *Folsomia candida* (Willem)

Animal Group: Phylum: Arthropoda; Class: Entognatha or Insecta

Details on Test Organisms: Juvenile collembolans (9-12 days old) were taken from stock originally obtained from Biologische Bundesanstalt (BBA; Berlin-Dahlem) in May 2000. The collembolans were reared in the laboratory under ambient conditions.

Twelve days before test initiation, well-fed collembolans of a uniform age were obtained by transferring egg clusters from the breeding containers to fresh containers with fresh substrate. After 72 hours, the egg clusters were removed and the hatched juveniles were fed granulated dry yeast. After nine days, the juveniles were collected and ten were introduced to each test vessel using an exhaustor.

B. STUDY DESIGN AND METHODS

Study Type: Laboratory
Test Duration Type: Reproduction
Limit Test: No
Total Exposure Duration: 28 days
Post-Exposure Observation Period: N/A
Remarks: N/A

Test Environmental Conditions:

Controlled environment room

Temperature: 19.4 to 22.0°C

Photoperiod and Lighting: 16L:8D, provided by artificial light (LumiLux L58W) at an intensity of 540 lux.

Artificial Soil: Composition was 5% sphagnum peat (finely ground); 20% kaolin clay (kaolinite content >30%); 0.3% calcium carbonate; 74.7% industrial quartz sand (Millisil W3, composed of >50% fine sand with particles between 50 and 200 micron); and deionized water. The artificial soil components for each treatment group were mixed twice for two minutes using a KitchenAid mixer at 240 rotations/minute. Two days before test initiation, the dry soil was moistened by adding DI water to adjust the water content to 40 to 60% of the maximum water holding capacity.

Max water holding capacity (WHC) was 41.7 g/100 g soil dry wt; water content (g/100 g soil dw) was 24.9 to 25.0 (~ 60% of WHC) at test initiation and 24.2 to 24.7 (58-59% of WHC) at test termination; pH was 6.03 to 6.17 at test initiation, and 5.82 to 5.97 at test termination.

Feeding: The collembolans were fed granulated dry yeast (2 mg per test vessel) at test initiation and after 14 days.

Breeding and Holding: Collembolans were bred in a Bellaplast plastic vessel containing plaster of Paris, activated charcoal, and water (8:1:9) under a 16L:8D photoperiod at 20±2°C.

Nominal Concentrations: 0 (solvent control), 8.2, 14.7, 26.5, 47.6, 85.7, 154.3, 277.8, and 500 mg a.i./kg soil dry weight (dw)

Test Units: Glass containers (150-mL), covered with a lid. The test vessels were randomly positioned in a controlled environment test room. The

Test Design:

positions were re-randomized on a weekly basis and the vessels briefly opened twice a week for aeration.

Juvenile collembolans were exposed to a solvent control and 8 test concentrations via treated artificial sediment for 28 days. The toxic standard boric acid was also included. Eight biological replicates were maintained in the control and four replicates were allocated to each treatment, with 10 collembolans in each replicate vessel. After 28 days, parental and juvenile collembolan abundance and adult survival were determined, in addition to observations on physiological and pathological symptoms or behavioral changes.

III. APPLICANT'S REPORTED RESULTS AND DISCUSSION

Exposure Duration: 28 days
Endpoint(s): NOAEC, LOAEC, EC₅₀ and LC₅₀
Effect Concentration: LC₅₀: 375.5 mg a.i./kg dry wt soil
EC₅₀: 425.3 mg a.i./kg dry wt soil
NOAEC: 154.3 mg a.i./kg dry wt soil
LOAEC: 277.8 mg a.i./kg dry wt soil
Basis for Concentration: Nominal
Effect Concentration Type: Active ingredient
Basis for Effect: Survival and reproduction

Details on Applicant-Provided Results:

All collembolans in the control and treatment groups exhibited similar behavior during the test. Survival in the solvent control averaged 96%. Survival ranged from 93 to 100% across all treatment groups except for the 277.8 and 500 mg ai/kg dw soil groups where survival averaged 75 and 28%, respectively. The number of juveniles produced averaged 700 in the solvent control. Juvenile production ranged from 681 to 705 across all treatment groups except for the 277.8 and 500 mg ai/kg dw soil groups where juvenile production averaged 454 and 310, respectively.

Test concentration (mg a.i./kg dw soil)	Day 28	
	Adult survival (%), mean ± standard deviation	No. of juveniles, mean ± standard deviation
Solvent Control	96 ± 5	700 ± 81.4
8.2	98 ± 5	699 ± 105.4
14.7	93 ± 5	705 ± 128.9
26.5	100 ± 0	684 ± 118.5
47.6	95 ± 6	693 ± 38.6
85.7	98 ± 5	681 ± 59.3
154.3	98 ± 5	690 ± 150
277.8	75 ± 13	454 ± 91.2
500	28 ± 17	310 ± 110.1

Applicant-Reported Statistics and Error Estimates

The solvent control was compared to the exposed treatment groups using the Multiple Sequentially-Rejective Fisher Test after a Bonferroni-Holm and Williams t-test. The survival data were analyzed via the Trimmed Spearman-Kärber test. The EC₅₀ was calculated using Probit analysis via linear maximum likelihood regression. The program ToxRat Professional 3.1.0 (2015) was used for the calculations.

IV. OVERALL REMARKS, ATTACHMENTS

The applicant submitted a full study report (PDF document), and an OECD-formatted summary document.

V. PRIMARY REVIEWER'S ANALYSIS AND CONCLUSIONS

Reviewer's Statistical Verification:

Adult survival and no. of juveniles were analyzed using CETIS statistical software version 1.8.7.12 with database backend settings implemented by EFED on 10/20/2015 (see **Appendix I** for CETIS® summary and analytical reports). No EPA guidance currently exists for this type of toxicity study, so the reviewer used the 21-day chronic daphnid template in CETIS for data entry and analysis. All treatment data were compared to the solvent control, because a negative control was not included.

Data were tested for normality using the Shapiro-Wilk's test ($\alpha = 0.01$) and for homogeneity of variance using Levene's test or Bartlett's test ($\alpha = 0.01$). Adult survival data did not pass the tests for normality and homogeneity of variance, and were subsequently analyzed using the Mann-Whitney test as survival data exhibited a non-monotonic response pattern. Number of offspring data met assumptions for parametric tests, so data were analyzed using analysis of variance (ANOVA) followed by Dunnett's multiple comparison test.

The reviewer attempted linear regression to determine the LC₅₀ value for adult survival. However, the resulting value was greater than the highest test concentration, the upper confidence limit was substantially higher than the highest test concentration, and there was significant lack of fit to the model. Therefore, the reviewer used the Trimmed Spearman-Kärber method to estimate the LC₅₀ value. The EC₅₀ value based on number of offspring data was determined using nonlinear regression (Probit).

All analyses were conducted using the nominal test concentrations, as test concentrations were not analytically verified; all tests were performed with $\alpha = 0.05$.

Survival:

LC ₅₀ : 386 mg a.i./kg dw soil	95% C.I.: 343-434 mg a.i./kg dw soil
Slope: N/A	95% C.I.: N/A
NOAEC: 154.3 mg a.i./kg dw soil	LOAEC: 277.8 mg a.i./kg dw soil

No. of Offspring:

EC ₅₀ : 426 mg a.i./kg dw soil	95% C.I.: 346-N/A mg a.i./kg dw soil
Slope: 0.75	95% C.I.: 0.38-1.13
NOAEC: 154.3 mg a.i./kg dw soil	LOAEC: 277.8 mg a.i./kg dw soil

Reviewer's Comments:

The reviewer's and study author's results were in general agreement. The reviewer and the study author both used the Trimmed Spearman-Kärber test for determination of the LC₅₀ value. However, the reviewer used nonlinear regression for the calculation of the EC₅₀ value, while the study author used linear regression. The reviewer's results are presented in the Executive Summary and Conclusions sections of this DER.

The in-life phase of the study was conducted from September 9 to October 7, 2015.

Reviewer's Conclusions:

Adult survival and reproduction were significantly affected in the two highest test concentrations. The overall NOAEC and LOAEC were 154.3 and 277.8 mg a.i./kg dw soil, respectively. The LC₅₀ was 386 mg a.i./kg dw soil and the EC₅₀ was 426 mg a.i./kg dw soil.

Results Synopsis:

Survival:

LC ₅₀ : 386 mg a.i./kg dw soil	95% C.I.: 343-434 mg a.i./kg dw soil
Slope: N/A	95% C.I.: N/A
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No. of Offspring:

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Slope: 0.75	95% C.I.: 0.38-1.13
NOAEC: 154.3 mg a.i./kg dw soil	LOAEC: 277.8 mg a.i./kg dw soil

Endpoint(s) affected: Survival and no. of offspring

EPA Classification: Supplemental (may be used quantitatively for risk estimation)

PMRA Classification: Fully reliable

APPENDIX I. CETIS® Summary and Analytical Reports

CETIS Summary Report

Report Date: 19 Jan-17 04:40 (p 1 of 2)
Test Code: 026200 49689112 | 10-2155-9014

OPPTS 850.1300 Chronic Invert (Daphnid)				BioChem Agrar	
Batch ID:	18-9150-1909	Test Type:	Chronic Daphnia (21-d)	Analyst:	
Start Date:	09 Sep-15	Protocol:	OPPTS 850.1300 Chronic Invert (Daphnid L	Diluent:	Acetone
Ending Date:	07 Oct-15	Species:	Folsomia candida	Brine:	
Duration:	28d 0h	Source:	Lab In-House Culture	Age:	
Sample ID:	02-6745-4533	Code:	49689112	Client:	CDM Smith - M. Wright
Sample Date:	09 Sep-15	Material:	Afidopyropen	Project:	
Receive Date:	07 Oct-15	Source:	BASF SE		
Sample Age:	NA	Station:			
Batch Note: 026200 49689112; 28-day study with artificial soil					
Sample Note: 026200 49689112; 28-day study with artificial soil					

Comparison Summary							
Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
06-4404-0023	F0 Survival	277.8	500	372.7	NA		Jonckheere-Terpstra Step-Down Test
12-3504-9044	F0 Survival	154.3	277.8	207	9.18%		Mann-Whitney U Two-Sample Test
10-0071-2173	n Live Offspring	154.3	277.8	207	22.6%		Dunnett Multiple Comparison Test
06-7176-3795	n Live Offspring	154.3	277.8	207	15.9%		Williams Multiple Comparison Test

Point Estimate Summary							
Analysis ID	Endpoint	Level	mg ai/kg	95% LCL	95% UCL	TU	Method
06-7141-6074	F0 Survival	LC5	44.7	7.94	85.3		Linear Regression (MLE)
		LC10	78.3	24.4	136		
		LC15	114	48.8	199		
		LC20	154	78.8	289		
		LC25	200	112	425		
		LC40	382	217	1380		
		LC50	565	298	3040		
13-9817-4350	F0 Survival	LC50	386	343	434		Trimmed Spearman-Kärber
21-0080-2155	n Live Offspring	IC5	124	N/A	179		Nonlinear Regression
		IC10	163	58.7	224		
		IC15	195	119	259		
		IC20	226	158	291		
		IC25	257	194	321		
		IC40	352	290	422		
		IC50	426	346	523		

CETIS Summary Report

Report Date: 19 Jan-17 04:40 (p 2 of 2)
 Test Code: 026200 49689112 | 10-2155-9014

OPPTS 850.1300 Chronic Invert (Daphnid)

BioChem Agrar

F0 Survival Summary

C-mg ai/kg	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Solvent Blank	8	0.963	0.919	1	0.9	1	0.0183	0.0518	5.38%	0.0%
8.2		4	0.975	0.895	1	0.9	1	0.025	0.05	5.13%	-1.3%
14.7		4	0.925	0.845	1	0.9	1	0.025	0.05	5.41%	3.9%
26.5		4	1	1	1	1	1	0	0	0.0%	-3.9%
47.6		4	0.95	0.858	1	0.9	1	0.0289	0.0577	6.08%	1.3%
85.7		4	0.975	0.895	1	0.9	1	0.025	0.05	5.13%	-1.3%
154.3		4	0.975	0.895	1	0.9	1	0.025	0.05	5.13%	-1.3%
277.8		4	0.75	0.545	0.955	0.6	0.9	0.0645	0.129	17.2%	22.1%
500		4	0.275	0.00325	0.547	0.1	0.5	0.0854	0.171	62.1%	71.4%

n Live Offspring Summary

C-mg ai/kg	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Solvent Blank	8	700	632	768	604	829	28.8	81.4	11.6%	0.0%
8.2		4	699	531	866	587	796	52.7	105	15.1%	0.23%
14.7		4	705	500	910	517	793	64.4	129	18.3%	-0.7%
26.5		4	684	495	872	574	790	59.3	119	17.3%	2.41%
47.6		4	693	631	754	655	728	19.3	38.6	5.57%	1.09%
85.7		4	681	587	775	611	754	29.6	59.3	8.71%	2.77%
154.3		4	690	452	929	496	825	75	150	21.7%	1.45%
277.8		4	454	309	599	338	549	45.6	91.2	20.1%	35.2%
500		4	310	135	485	189	444	55.1	110	35.5%	55.7%

F0 Survival Detail

C-mg ai/kg	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8
0	Solvent Blank	0.9	1	0.9	1	1	0.9	1	1
8.2		0.9	1	1	1				
14.7		0.9	0.9	1	0.9				
26.5		1	1	1	1				
47.6		1	0.9	0.9	1				
85.7		1	0.9	1	1				
154.3		0.9	1	1	1				
277.8		0.8	0.9	0.7	0.6				
500		0.1	0.5	0.3	0.2				

n Live Offspring Detail

C-mg ai/kg	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8
0	Solvent Blank	653	727	680	604	795	705	829	610
8.2		631	796	781	587				
14.7		784	727	793	517				
26.5		574	790	782	588				
47.6		728	724	655	664				
85.7		667	611	692	754				
154.3		825	650	496	790				
277.8		498	549	431	338				
500		261	444	347	189				

CETIS Analytical Report

Report Date: 19 Jan-17 04:38 (p 1 of 8)
Test Code: 026200 49689112 | 10-2155-9014

OPPTS 850.1300 Chronic Invert (Daphnid)

BioChem Agrar

Analysis ID: 12-3504-9044 **Endpoint:** F0 Survival **CETIS Version:** CETISv1.8.7
Analyzed: 19 Jan-17 4:36 **Analysis:** Nonparametric-Two Sample **Official Results:** Yes
Batch ID: 18-9150-1909 **Test Type:** Chronic Daphnia (21-d) **Analyst:**
Start Date: 09 Sep-15 **Protocol:** OPPTS 850.1300 Chronic Invert (Daphnid L **Diluent:** Acetone
Ending Date: 07 Oct-15 **Species:** Folsomia candida **Brine:**
Duration: 28d 0h **Source:** Lab In-House Culture **Age:**

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Untransformed	NA	C > T	NA	NA	9.18%	154.3	277.8	207	

Mann-Whitney U Two-Sample Test

Control	vs	C-mg ai/kg	Test Stat	Critical	Ties	DF	P-Value	P-Type	Decision(α:5%)
Solvent Blank		8.2	14	NA	2	10	0.5939	Exact	Non-Significant Effect
		14.7	22	NA	2	10	0.2727	Exact	Non-Significant Effect
		26.5	10	NA	1	10	0.9818	Exact	Non-Significant Effect
		47.6	18	NA	2	10	0.4242	Exact	Non-Significant Effect
		85.7	14	NA	2	10	0.5939	Exact	Non-Significant Effect
		154.3	14	NA	2	10	0.5939	Exact	Non-Significant Effect
		277.8*	30.5	NA	1	10	<0.0001	Exact	Significant Effect
		500*	32	NA	0	10	0.0020	Exact	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	1.77875	0.2223437	8	35.1	<0.0001	Significant Effect
Error	0.19625	0.006330645	31			
Total	1.975		39			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Mod Levene Equality of Variance	2.37	3.15	0.0405	Equal Variances
Variances	Levene Equality of Variance	3.99	3.15	0.0024	Unequal Variances
Distribution	Shapiro-Wilk W Normality	0.918	0.924	0.0067	Non-normal Distribution

F0 Survival Summary

C-mg ai/kg	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Solvent Blank	8	0.962	0.919	1	1	0.9	1	0.0183	5.38%	0.0%
8.2		4	0.975	0.895	1	1	0.9	1	0.025	5.13%	-1.3%
14.7		4	0.925	0.845	1	0.9	0.9	1	0.025	5.41%	3.9%
26.5		4	1	1	1	1	1	1	0	0.0%	-3.9%
47.6		4	0.95	0.858	1	0.95	0.9	1	0.0289	6.08%	1.3%
85.7		4	0.975	0.895	1	1	0.9	1	0.025	5.13%	-1.3%
154.3		4	0.975	0.895	1	1	0.9	1	0.025	5.13%	-1.3%
277.8		4	0.75	0.545	0.955	0.75	0.6	0.9	0.0645	17.2%	22.1%
500		4	0.275	0.00325	0.547	0.25	0.1	0.5	0.0854	62.1%	71.4%

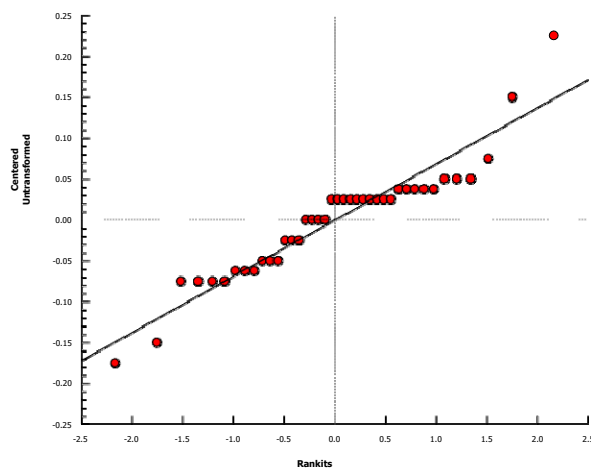
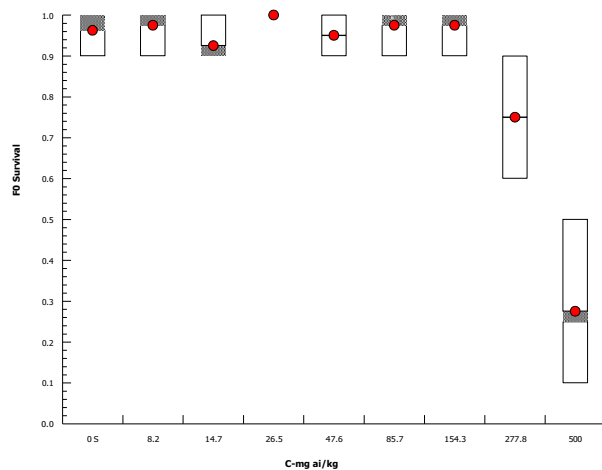
OPPTS 850.1300 Chronic Invert (Daphnid)

BioChem Agrar

Analysis ID: 12-3504-9044 Endpoint: F0 Survival
Analyzed: 19 Jan-17 4:36 Analysis: Nonparametric-Two Sample

CETIS Version: CETISv1.8.7
Official Results: Yes

Graphics



CETIS Analytical Report

Report Date: 19 Jan-17 04:38 (p 3 of 8)
Test Code: 026200 49689112 | 10-2155-9014

OPPTS 850.1300 Chronic Invert (Daphnid)

BioChem Agrar

Analysis ID: 06-4404-0023 **Endpoint:** F0 Survival **CETIS Version:** CETISv1.8.7
Analyzed: 19 Jan-17 4:36 **Analysis:** Nonparametric-Control vs Ord. Treatments **Official Results:** Yes
Batch ID: 18-9150-1909 **Test Type:** Chronic Daphnia (21-d) **Analyst:**
Start Date: 09 Sep-15 **Protocol:** OPPTS 850.1300 Chronic Invert (Daphnid L **Diluent:** Acetone
Ending Date: 07 Oct-15 **Species:** Folsomia candida **Brine:**
Duration: 28d 0h **Source:** Lab In-House Culture **Age:**

Data Transform	Zeta	Alt Hyp	Trials	Seed	NOEL	LOEL	TOEL	TU
Untransformed	NA	C > T	NA	NA	277.8	500	372.7	

Jonckheere-Terpstra Step-Down Test

Control	vs	C-mg ai/kg	Test Stat	Critical	Ties	DF	P-Value	P-Type	Decision(α:5%)
Solvent Blank		8.2	-0.496	1.64	2	-2	0.6962	Asymp	Non-Significant Effect
		14.7	0.965	1.64	2	-2	0.6962	Asymp	Non-Significant Effect
		26.5	-0.513	1.64	2	-2	0.6962	Asymp	Non-Significant Effect
		47.6	0	1.64	2	-2	0.6870	Asymp	Non-Significant Effect
		85.7	-0.297	1.64	2	-2	0.6870	Asymp	Non-Significant Effect
		154.3	-0.487	1.64	2	-2	0.6870	Asymp	Non-Significant Effect
		277.8	1.48	1.64	2	-2	0.0696	Asymp	Non-Significant Effect
		500*	3.08	1.64	2	-2	0.0010	Asymp	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	1.77875	0.2223437	8	35.1	<0.0001	Significant Effect
Error	0.19625	0.006330645	31			
Total	1.975		39			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Mod Levene Equality of Variance	2.37	3.15	0.0405	Equal Variances
Variances	Levene Equality of Variance	3.99	3.15	0.0024	Unequal Variances
Distribution	Shapiro-Wilk W Normality	0.918	0.924	0.0067	Non-normal Distribution

F0 Survival Summary

C-mg ai/kg	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Solvent Blank	8	0.962	0.919	1	1	0.9	1	0.0183	5.38%	0.0%
8.2		4	0.975	0.895	1	1	0.9	1	0.025	5.13%	-1.3%
14.7		4	0.925	0.845	1	0.9	0.9	1	0.025	5.41%	3.9%
26.5		4	1	1	1	1	1	1	0	0.0%	-3.9%
47.6		4	0.95	0.858	1	0.95	0.9	1	0.0289	6.08%	1.3%
85.7		4	0.975	0.895	1	1	0.9	1	0.025	5.13%	-1.3%
154.3		4	0.975	0.895	1	1	0.9	1	0.025	5.13%	-1.3%
277.8		4	0.75	0.545	0.955	0.75	0.6	0.9	0.0645	17.2%	22.1%
500		4	0.275	0.00325	0.547	0.25	0.1	0.5	0.0854	62.1%	71.4%

OPPTS 850.1300 Chronic Invert (Daphnid)

BioChem Agrar

Analysis ID: 06-4404-0023

Endpoint: F0 Survival

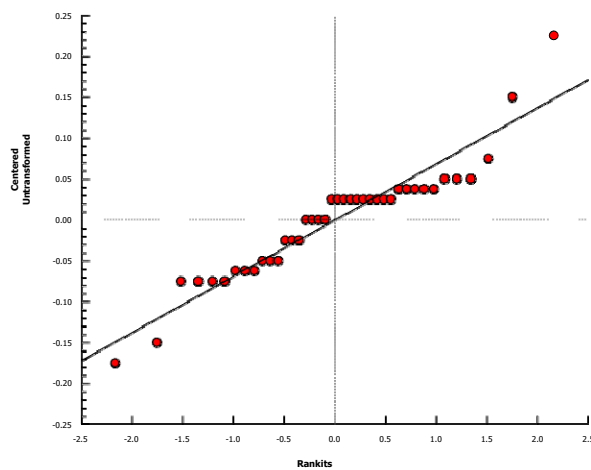
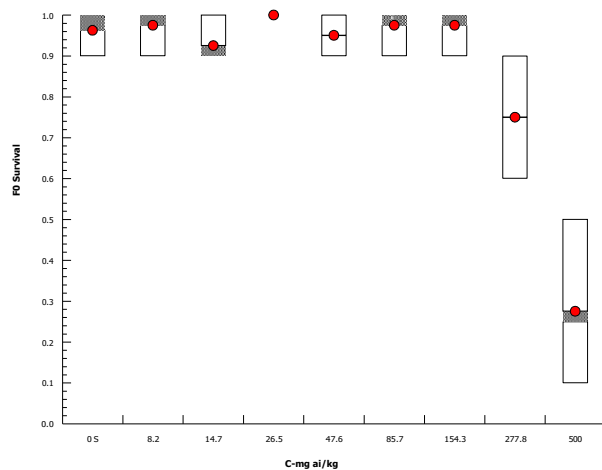
CETIS Version: CETISv1.8.7

Analyzed: 19 Jan-17 4:36

Analysis: Nonparametric-Control vs Ord. Treatments

Official Results: Yes

Graphics



CETIS Analytical Report

Report Date: 19 Jan-17 04:38 (p 5 of 8)
Test Code: 026200 49689112 | 10-2155-9014

OPPTS 850.1300 Chronic Invert (Daphnid)

BioChem Agrar

Analysis ID:	10-0071-2173	Endpoint:	n Live Offspring	CETIS Version:	CETISv1.8.7
Analyzed:	19 Jan-17 4:36	Analysis:	Parametric-Control vs Treatments	Official Results:	Yes
Batch ID:	18-9150-1909	Test Type:	Chronic Daphnia (21-d)	Analyst:	
Start Date:	09 Sep-15	Protocol:	OPPTS 850.1300 Chronic Invert (Daphnid L	Diluent:	Acetone
Ending Date:	07 Oct-15	Species:	Folsomia candida	Brine:	
Duration:	28d 0h	Source:	Lab In-House Culture	Age:	

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Untransformed	NA	C > T	NA	NA	22.6%	154.3	277.8	207	

Dunnett Multiple Comparison Test

Control	vs	C-mg ai/kg	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Solvent Blank		8.2	0.0263	2.56	158	10	0.9350	CDF	Non-Significant Effect
		14.7	-0.0789	2.56	158	10	0.9513	CDF	Non-Significant Effect
		26.5	0.273	2.56	158	10	0.8804	CDF	Non-Significant Effect
		47.6	0.123	2.56	158	10	0.9164	CDF	Non-Significant Effect
		85.7	0.314	2.56	158	10	0.8691	CDF	Non-Significant Effect
		154.3	0.164	2.56	158	10	0.9076	CDF	Non-Significant Effect
		277.8*	3.99	2.56	158	10	0.0014	CDF	Significant Effect
		500*	6.32	2.56	158	10	<0.0001	CDF	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	666056.5	83257.06	8	8.18	<0.0001	Significant Effect
Error	315402.6	10174.28	31			
Total	981459.1		39			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	6.24	20.1	0.6206	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.955	0.924	0.1127	Normal Distribution

n Live Offspring Summary

C-mg ai/kg	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Solvent Blank	8	700	632	768	693	604	829	28.8	11.6%	0.0%
8.2		4	699	531	866	706	587	796	52.7	15.1%	0.23%
14.7		4	705	500	910	756	517	793	64.4	18.3%	-0.7%
26.5		4	684	495	872	685	574	790	59.3	17.3%	2.41%
47.6		4	693	631	754	694	655	728	19.3	5.57%	1.09%
85.7		4	681	587	775	680	611	754	29.6	8.71%	2.77%
154.3		4	690	452	929	720	496	825	75	21.7%	1.45%
277.8		4	454	309	599	465	338	549	45.6	20.1%	35.2%
500		4	310	135	485	304	189	444	55.1	35.5%	55.7%

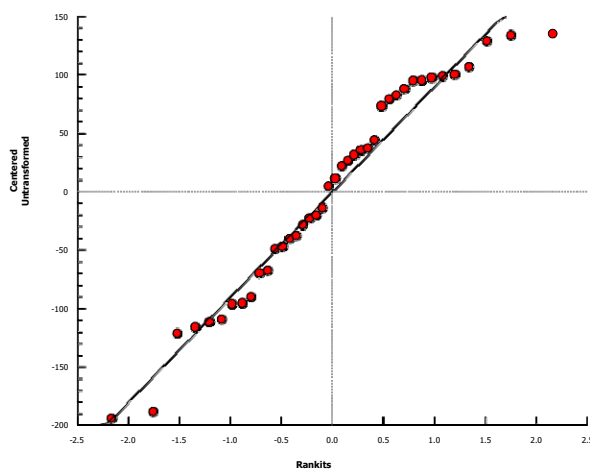
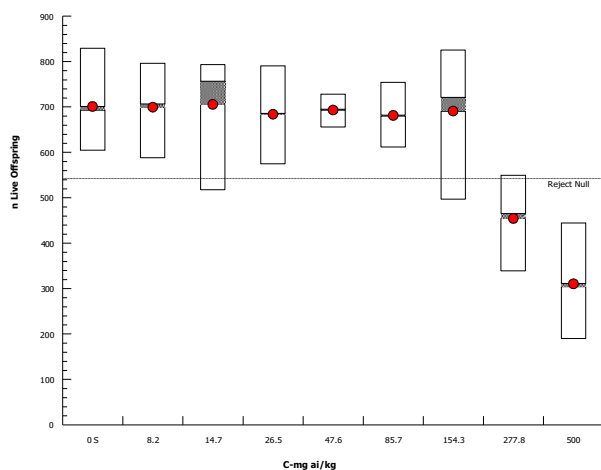
OPPTS 850.1300 Chronic Invert (Daphnid)

BioChem Agrar

Analysis ID: 10-0071-2173 Endpoint: nLive Offspring
Analyzed: 19 Jan-17 4:36 Analysis: Parametric-Control vs Treatments

CETIS Version: CETISv1.8.7
Official Results: Yes

Graphics



CETIS Analytical Report

Report Date: 19 Jan-17 04:38 (p 7 of 8)
Test Code: 026200 49689112 | 10-2155-9014

OPPTS 850.1300 Chronic Invert (Daphnid)

BioChem Agrar

Analysis ID: 06-7176-3795 **Endpoint:** n Live Offspring **CETIS Version:** CETISv1.8.7
Analyzed: 19 Jan-17 4:36 **Analysis:** Parametric-Control vs Ord.Treatments **Official Results:** Yes
Batch ID: 18-9150-1909 **Test Type:** Chronic Daphnia (21-d) **Analyst:**
Start Date: 09 Sep-15 **Protocol:** OPPTS 850.1300 Chronic Invert (Daphnid L **Diluent:** Acetone
Ending Date: 07 Oct-15 **Species:** Folsomia candida **Brine:**
Duration: 28d 0h **Source:** Lab In-House Culture **Age:**

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Untransformed	NA	C > T	NA	NA	15.9%	154.3	277.8	207	

Williams Multiple Comparison Test

Control	vs	C-mg ai/kg	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Solvent Blank		8.2	0.0263	1.7	105	10	>0.05	CDF	Non-Significant Effect
		14.7	-0.0263	1.76	109	10	>0.05	CDF	Non-Significant Effect
		26.5	0.273	1.78	110	10	>0.05	CDF	Non-Significant Effect
		47.6	0.198	1.79	110	10	>0.05	CDF	Non-Significant Effect
		85.7	0.314	1.79	111	10	>0.05	CDF	Non-Significant Effect
		154.3	0.239	1.79	111	10	>0.05	CDF	Non-Significant Effect
		277.8*	3.99	1.8	111	10	<0.05	CDF	Significant Effect
		500*	6.32	1.8	111	10	<0.05	CDF	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	666056.5	83257.06	8	8.18	<0.0001	Significant Effect
Error	315402.6	10174.28	31			
Total	981459.1		39			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	6.24	20.1	0.6206	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.955	0.924	0.1127	Normal Distribution

n Live Offspring Summary

C-mg ai/kg	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Solvent Blank	8	700	632	768	693	604	829	28.8	11.6%	0.0%
8.2		4	699	531	866	706	587	796	52.7	15.1%	0.23%
14.7		4	705	500	910	756	517	793	64.4	18.3%	-0.7%
26.5		4	684	495	872	685	574	790	59.3	17.3%	2.41%
47.6		4	693	631	754	694	655	728	19.3	5.57%	1.09%
85.7		4	681	587	775	680	611	754	29.6	8.71%	2.77%
154.3		4	690	452	929	720	496	825	75	21.7%	1.45%
277.8		4	454	309	599	465	338	549	45.6	20.1%	35.2%
500		4	310	135	485	304	189	444	55.1	35.5%	55.7%

OPPTS 850.1300 Chronic Invert (Daphnid)

BioChem Agrar

Analysis ID: 06-7176-3795

Endpoint: nLive Offspring

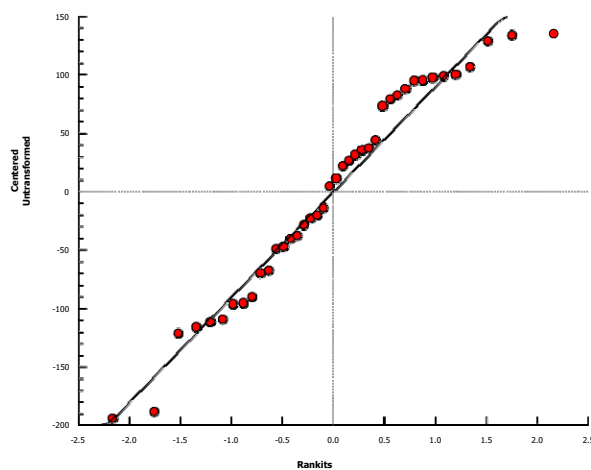
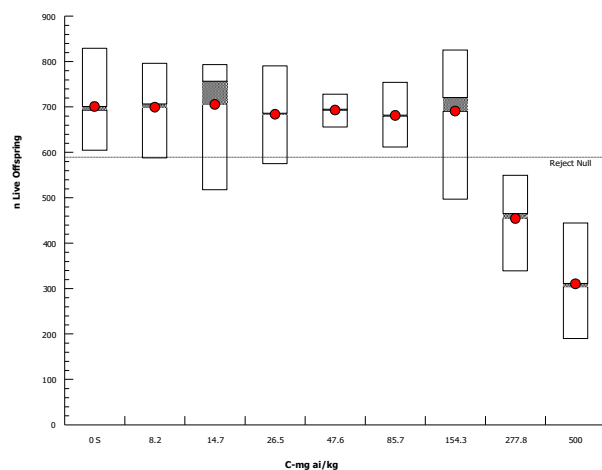
CETIS Version: CETISv1.8.7

Analyzed: 19 Jan-17 4:36

Analysis: Parametric-Control vs Ord.Treatments

Official Results: Yes

Graphics



CETIS Analytical Report

Report Date: 19 Jan-17 04:39 (p 1 of 2)
Test Code: 026200 49689112 | 10-2155-9014

OPPTS 850.1300 Chronic Invert (Daphnid) BioChem Agrar

Analysis ID: 06-7141-6074	Endpoint: F0 Survival	CETIS Version: CETISv1.8.7
Analyzed: 19 Jan-17 4:36	Analysis: Linear Regression (MLE)	Official Results: Yes
Batch ID: 18-9150-1909	Test Type: Chronic Daphnia (21-d)	Analyst:
Start Date: 09 Sep-15	Protocol: OPPTS 850.1300 Chronic Invert (Daphnid L	Diluent: Acetone
Ending Date: 07 Oct-15	Species: Folsomia candida	Brine:
Duration: 28d 0h	Source: Lab In-House Culture	Age:

Linear Regression Options						
Model Function	Threshold Option	Threshold	Optimized	Pooled	Het Corr	Weighted
Log-Normal [NED=A+B*log(X)]	Zero Threshold	0	No	No	Yes	Yes

Regression Summary										
Iters	LL	AICc	BIC	Mu	Sigma	Adj R2	F Stat	Critical	P-Value	Decision(α:5%)
9	-98.3	201	204	2.75	0.67	0.371	14.8	2.51	0.0000	Significant Lack of Fit

Point Estimates			
Level	mg ai/kg	95% LCL	95% UCL
LC5	44.7	7.94	85.3
LC10	78.3	24.4	136
LC15	114	48.8	199
LC20	154	78.8	289
LC25	200	112	425
LC40	382	217	1380
LC50	565	298	3040

Regression Parameters							
Parameter	Estimate	Std Error	95% LCL	95% UCL	t Stat	P-Value	Decision(α:5%)
Slope	1.49	0.397	0.682	2.3	3.76	0.0007	Significant Parameter
Intercept	-4.11	0.895	-5.94	-2.28	-4.59	<0.0001	Significant Parameter

ANOVA Table						
Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Model	58.73253	58.73253	1	19.3	0.0001	Significant
Lack of Fit	71.80667	11.96778	6	14.8	<0.0001	Significant
Pure Error	19.47249	0.811354	24			
Residual	91.27916	3.042639	30			

Residual Analysis					
Attribute	Method	Test Stat	Critical	P-Value	Decision(α:5%)
Goodness-of-Fit	Pearson Chi-Sq GOF	91.3	43.8	<0.0001	Significant Heterogeneity
	Likelihood Ratio GOF	59.9	43.8	0.0010	Significant Heterogeneity
Variances	Mod Levene Equality of Variance	0.553	2.42	0.7857	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.839	0.934	0.0002	Non-normal Distribution
	Anderson-Darling A2 Normality	2.03	2.49	<0.0001	Non-normal Distribution

F0 Survival Summary			Calculated Variate(A/B)								
C-mg ai/kg	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect	A	B
8.2		4	0.975	0.9	1	0.025	0.05	5.13%	0.0%	39	40
14.7		4	0.925	0.9	1	0.025	0.05	5.41%	5.13%	37	40
26.5		4	1	1	1	0	0	0.0%	-2.56%	40	40
47.6		4	0.95	0.9	1	0.0289	0.0577	6.08%	2.56%	38	40
85.7		4	0.975	0.9	1	0.025	0.05	5.13%	0.0%	39	40
154.3		4	0.975	0.9	1	0.025	0.05	5.13%	0.0%	39	40
277.8		4	0.75	0.6	0.9	0.0645	0.129	17.2%	23.1%	30	40
500		4	0.275	0.1	0.5	0.0854	0.171	62.1%	71.8%	11	40

OPPTS 850.1300 Chronic Invert (Daphnid)

BioChem Agrar

Analysis ID: 06-7141-6074

Endpoint: F0 Survival

CETIS Version: CETISv1.8.7

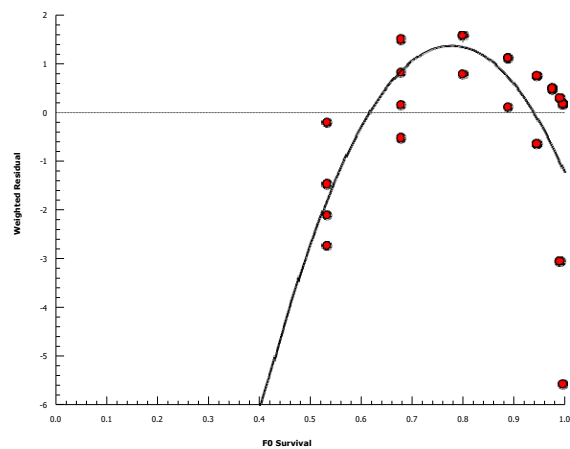
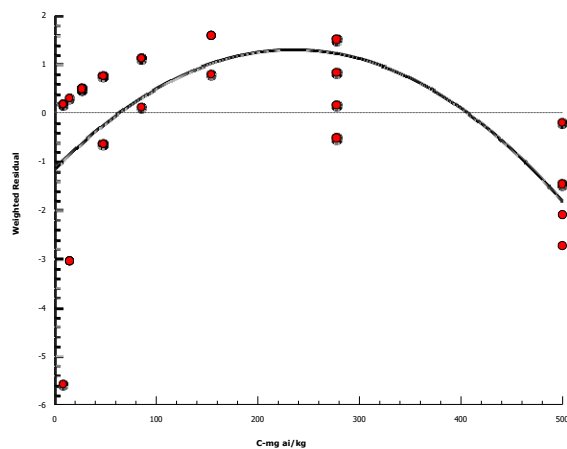
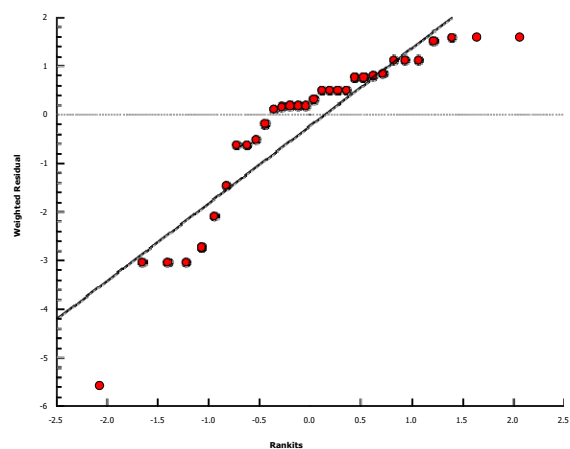
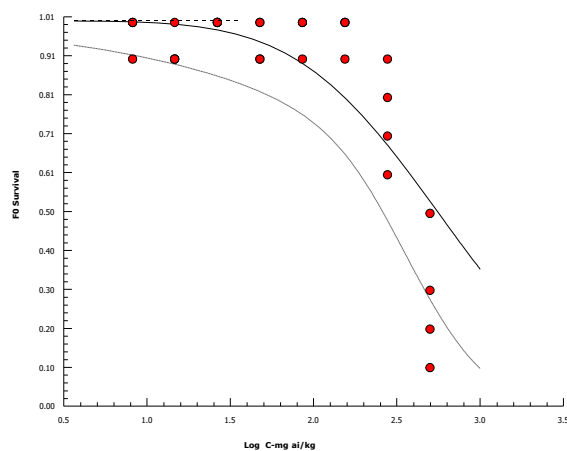
Analyzed: 19 Jan-17 4:36

Analysis: Linear Regression (MLE)

Official Results: Yes

Graphics

Log-Normal [NED=A+B*log(X)]



CETIS Analytical Report

Report Date: 19 Jan-17 04:39 (p 1 of 2)
Test Code: 026200 49689112 | 10-2155-9014

OPPTS 850.1300 Chronic Invert (Daphnid)			BioChem Agrar		
Analysis ID:	21-0080-2155	Endpoint:	nLive Offspring	CETIS Version:	CETISv1.8.7
Analyzed:	19 Jan-17 4:36	Analysis:	Nonlinear Regression	Official Results:	Yes
Batch ID:	18-9150-1909	Test Type:	Chronic Daphnia (21-d)	Analyst:	
Start Date:	09 Sep-15	Protocol:	OPPTS 850.1300 Chronic Invert (Daphnid L	Diluent:	Acetone
Ending Date:	07 Oct-15	Species:	Folsomia candida	Brine:	
Duration:	28d 0h	Source:	Lab In-House Culture	Age:	

Non-Linear Regression Options				
Model Function	X Transform	Y Transform	Weighting Function	PTBS Function
3P Cumulative Log-Normal EV [Y=A*(1- Φ(log(X/D)/C))]	None	None	Normal [W=1]	Off [Y*=Y]

Regression Summary									
Iters	Log LL	AICc	BIC	Adj R2	Optimize	F Stat	Critical	P-Value	Decision(α:5%)
11	-201	408	413	0.6370	Yes	0.371	2.41	0.8920	Non-Significant Lack of Fit

Point Estimates			
Level	mg ai/kg	95% LCL	95% UCL
IC5	124	N/A	179
IC10	163	58.7	224
IC15	195	119	259
IC20	226	158	291
IC25	257	194	321
IC40	352	290	422
IC50	426	346	523

Regression Parameters							
Parameter	Estimate	Std Error	95% LCL	95% UCL	t Stat	P-Value	Decision(α:5%)
A	700	18.8	663	737	37.2	<0.0001	Significant Parameter
C	0.751	0.191	0.377	1.13	3.94	0.0003	Significant Parameter
D	426	45.6	336	515	9.34	<0.0001	Significant Parameter

ANOVA Table						
Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Model	643437.6	643437.6	1	70.4	<0.0001	Significant
Lack of Fit	22618.82	3769.804	6	0.371	0.8920	Non-Significant
Pure Error	315402.6	10174.28	31			
Residual	338021.4	9135.715	37			

Residual Analysis					
Attribute	Method	Test Stat	Critical	P-Value	Decision(α :5%)
Variances	Bartlett Equality of Variance	6.24	15.5	0.6206	Equal Variances
	Mod Levene Equality of Variance	1.16	2.25	0.3559	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.982	0.945	0.7618	Normal Distribution
	Anderson-Darling A2 Normality	0.245	2.49	0.7875	Normal Distribution

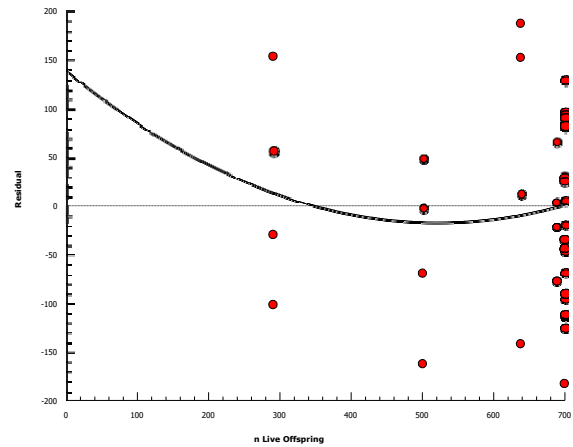
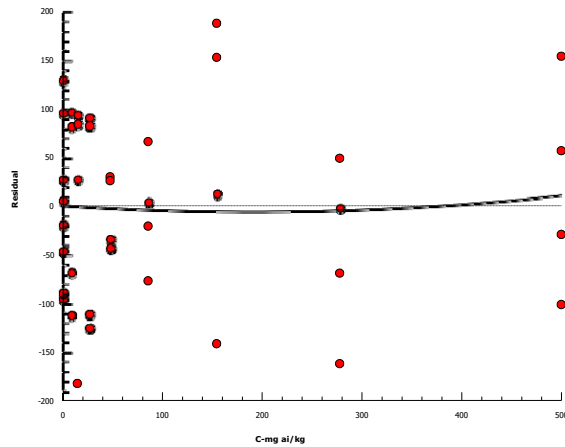
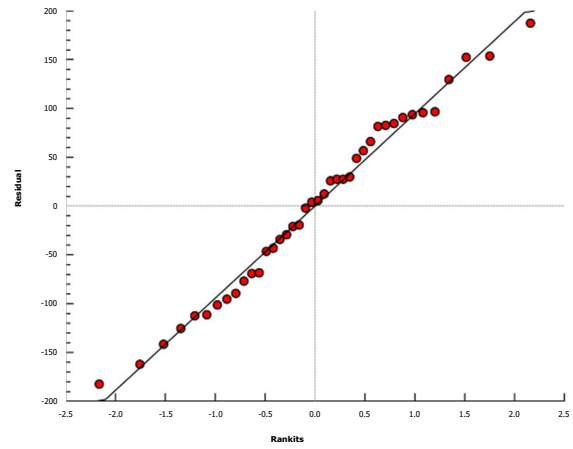
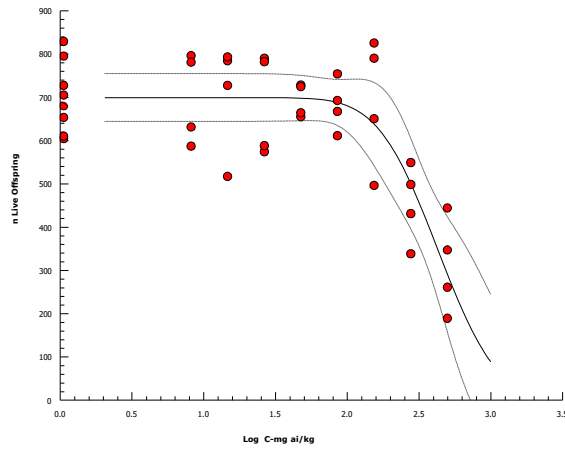
n Live Offspring Summary			Calculated Variate						
C-mg ai/kg	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Solvent Blank	8	700	604	829	28.8	81.4	11.6%	0.0%
8.2		4	699	587	796	52.7	105	15.1%	0.23%
14.7		4	705	517	793	64.4	129	18.3%	-0.7%
26.5		4	684	574	790	59.3	119	17.3%	2.41%
47.6		4	693	655	728	19.3	38.6	5.57%	1.09%
85.7		4	681	611	754	29.6	59.3	8.71%	2.77%
154.3		4	690	496	825	75	150	21.7%	1.45%
277.8		4	454	338	549	45.6	91.2	20.1%	35.2%
500		4	310	189	444	55.1	110	35.5%	55.7%

OPPTS 850.1300 Chronic Invert (Daphnid)

BioChem Agrar

Analysis ID: 21-0080-2155 Endpoint: nLive Offspring
Analyzed: 19 Jan-17 4:36 Analysis: Nonlinear RegressionCETIS Version: CETISv1.8.7
Official Results: Yes

Graphics

3P Cumulative Log-Normal EV [$Y=A*(1- \Phi(\log(X/D)/C))$]

CETIS Analytical Report

Report Date: 19 Jan-17 04:39 (p 1 of 1)
 Test Code: 026200 49689112 | 10-2155-9014

OPPTS 850.1300 Chronic Invert (Daphnid)

BioChem Agrar

Analysis ID: 13-9817-4350	Endpoint: F0 Survival	CETIS Version: CETISv1.8.7
Analyzed: 19 Jan-17 4:36	Analysis: Trimmed Spearman-Kärber	Official Results: Yes
Batch ID: 18-9150-1909	Test Type: Chronic Daphnia (21-d)	Analyst:
Start Date: 09 Sep-15	Protocol: OPPTS 850.1300 Chronic Invert (Daphnid L	Diluent: Acetone
Ending Date: 07 Oct-15	Species: Folsomia candida	Brine:
Duration: 28d 0h	Source: Lab In-House Culture	Age:

Trimmed Spearman-Kärber Estimates

Threshold Option	Threshold	Trim	Mu	Sigma	LC50	95% LCL	95% UCL
Control Threshold	0.0375	28.39%	2.59	0.0257	386	343	434

F0 Survival Summary

Calculated Variate(A/B)

C-mg ai/kg	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect	A	B
0	Solvent Blank	8	0.962	0.9	1	0.0183	0.0518	5.38%	0.0%	77	80
8.2		4	0.975	0.9	1	0.025	0.05	5.13%	-1.3%	39	40
14.7		4	0.925	0.9	1	0.025	0.05	5.41%	3.9%	37	40
26.5		4	1	1	1	0	0	0.0%	-3.9%	40	40
47.6		4	0.95	0.9	1	0.0289	0.0577	6.08%	1.3%	38	40
85.7		4	0.975	0.9	1	0.025	0.05	5.13%	-1.3%	39	40
154.3		4	0.975	0.9	1	0.025	0.05	5.13%	-1.3%	39	40
277.8		4	0.75	0.6	0.9	0.0645	0.129	17.2%	22.1%	30	40
500		4	0.275	0.1	0.5	0.0854	0.171	62.1%	71.4%	11	40

Graphics

